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Eared Dove (*Zenaida Auriculata*, Columbidae) as Host for St. Louis Encephalitis Virus (Flaviviridae, *Flavivirus*)

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ABSTRACT

St. Louis encephalitis virus (SLEV) is an emerging *Flavivirus* in South American countries. Its ecology and biological transmission cycles are scarcely known. Eared doves (*Zenaida auriculata*) have frequently been found infected by SLEV, and therefore, could be suspected as SLEV hosts. Thirty post-hatch-year eared doves were subcutaneously inoculated with the genotype V SLEV 78V-6507 viral strain and subsequently bled. No deaths or clinical signs of illness were observed in the inoculated doves. The viremia titers ranged from 2 to 5.5 $\log_{10}$ plaque-forming units (PFU)/mL during 1–7 days postinoculation (dpi), the highest being observed on the 4th dpi. Mosquitoes were collected using can traps baited with chicken and eared doves for comparison. A total of 2792 mosquitoes belonging to 5 species were collected. Ninety percent of the mosquitoes collected in eared dove-baited can traps were *Culex quinquefasciatus*. Statistical differences were not observed in either *Cx. quinquefasciatus* ($X^2 = 0.86$; df = 1; $p = 0.354$) or in *Cx. interfor* ($X^2 = 0.63$; df = 1; $p = 0.426$) mosquitoes collected in both chicken- and eared dove-baited can traps. Considering that eared doves were frequently found naturally infected by SLEV, that they developed viremia titers higher than the minimum infection threshold needed to infect *Cx. quinquefasciatus*, and that these mosquitoes also fed on eared doves, they could be considered competent hosts for SLEV. Key words: St. Louis encephalitis virus—Eared dove (*Zenaida auriculata*)—Avian host competence—*Flavivirus*—*Culex quinquefasciatus*.

INTRODUCTION

ST. LOUIS ENCEPHALITIS VIRUS (SLEV; *Flaviviridae*, *Flavivirus*) is widely distributed in the United States and Central and South America, being maintained in transmission cycles involving *Culex* mosquitoes and several bird species (Reisen 2003). Currently, SLEV is an important emerging arbovirosis in South America, with encephalitis cases reported in Argentina and Brazil (Spinsanti et al. 2003, Rocco et al. 2005, Diaz et al. 2006).

Based on serological data, SLEV is distributed in Argentina from the northern subtropical provinces to the southern temperate province of Rio Negro. Sporadic symptomatic cases of SLEV have been reported since 1964 (Sabattini et al. 1998). SLEV has been isolated from *Culex* mosquitoes, rodents, and febrile humans, but never from birds. Serological evidence of natural infection has been detected in horses, goats, cattle, and wild and domestic birds (Sabattini et al. 1998). In 2005, during an outbreak of SLEV in Córdoba Province (central Argentina), 47 laboratory-confirmed and 9 fatal cases were reported (Spinsanti et al. 2005). Moreover, 2 genotype III SLEV strains were also isolated from *Cx. quinquefasciatus* during the outbreak (Diaz et al. 2006).

The transmission cycles for SLEV mainte-

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nance in the temperate and subtropical areas of Argentina are scarcely known. *Cx. quinquefasciatus* has been found naturally infected with SLEV (Diaz et al. 2006) and was observed as a competent experimental vector for this virus (Mitchell et al. 1980), indicating its possible role as the main vector in Argentina. Based on serological studies by neutralization test carried out in temperate and subtropical areas in Argentina (Chaco, Córdoba, Corrientes, and Santa Fe provinces), numerous bird species belonging to Columbidae, Furnaridae, and Tyrannidae families have been found positive for SLEV (Monath et al. 1985, Diaz et al. 2005). More than 200 serum samples of house sparrows (*Passer domesticus*) were assayed by neutralization test against SLEV with no positive result (Monath et al. 1985). Adult house sparrows experimentally infected with SLEV produced viremia titers under the minimum infection threshold needed to infect *Cx. quinquefasciatus*, indicating these birds would not play an important role as amplifier hosts in Argentina (Sabattini et al. 1998).

On the other hand, picui ground doves (*Columbina picui*) and eared doves (*Zenaida auriculata*) (Columbidae) frequently had high antibody prevalence (10%–20%) (Sabattini et al. 1998), and therefore may be important hosts in the SLEV transmission cycle. The purpose of the current research was to determine if the eared dove is a competent host for SLEV by analyzing the viremia profiles and evaluating how attractive this dove is to host-seeking *Culex* mosquitoes.

MATERIALS AND METHODS

Birds and animal care

Eared doves were collected at Córdoba City University Campus (Argentina) using a ground grain-baited trap during spring. The Authorization was obtained from the Wildlife Division, Agencia Córdoba Ambiente, Córdoba, Argentina. Eared doves were maintained at the Virology Institute biosafety facilities under seminatural conditions (daylight period and temperature depending upon environmental conditions), and fed mixed grains ad libitum. After being collected, eared doves were bled and banded; 100 µL per bird was taken and stored at room temperature for 30 minutes to coagulate; the sera obtained were analyzed by hemagglutination inhibition assay (HIA) and plaque reduction neutralization test (PRNT) for antibodies against SLEV. Seronegative eared doves were used for the experiment, while those seropositive were released.

Viral strain

Eared doves were experimentally infected with the genotype V SLEV 78V-6507 strain, isolated from *Cx. quinquefasciatus* collected in 1978 in Esperanza, Santa Fe Province (eastern Argentina) (Mitchell et al. 1985), and kept at our Virology Institute. The viral suspension was prepared from a 10% dilution of infected suckling-mice brain in minimum essential medium (MEM) with Earle's salts and L-glutamine, 10% fetal calf serum (FCS), and 1% gentamicin, and centrifuged at 11,400*g* at 4°C for 30 minutes. The viral suspension was titrated by Vero cell plaque assay and 100-μL aliquots were stored at −80°C. The viral titer was expressed as plaque-forming units per milliliter (PFU/mL) (Diaz et al. 2003).

Viremia profile assays

Thirty post hatch-year eared doves were subcutaneously inoculated in the cervical region with 0.1 mL containing 300 PFU, keeping them in 3 groups of 10 individuals each (2 individuals per cage). Eared doves were easily stressed by handling, so to avoid physical damage, a different group was bled every day from the brachial vein using 28-gauge needles; i.e., each group was bled 3 times (every 72 hours) over a 9-day period. A control group with 10 individuals was not inoculated as a negative morbidity–mortality control. Inoculated eared doves were observed every 12 hours to detect any clinical signs of illness. Two hundred microliters of whole blood was diluted in 0.9 mL of refrigerated phosphate-buffered saline (PBS) with 10% FCS and 1% gentamicin to avoid bacterial contamination, centrifuged at 1500*g* for 15 minutes, and the supernatant stored at −80°C. Viremia titer was measured by plaque assay on Vero cells

and expressed as PFU/mL. Our detection threshold was 2 $\log_{10}$ PFU/mL.

Serology

To verify seroconversion in the inoculated eared doves against the SLEV 78V-6507 strain, all surviving individuals were bled 14 days postinoculation (dpi). The whole blood obtained was left at room temperature for 30 minutes to coagulate, then centrifuged to separate the serum, stored at −20°C, and heat-inactivated at 56°C for 30 minutes prior to testing. Sera were diluted 1:10 in MEM for the PRNT, and endpoint antibody titers determined using serial 2-fold dilutions.

Mosquito host preference studies

To evaluate how attractive *Z. auriculata* is to *Culex* mosquitoes, collections were carried out during 15 consecutive nights with baited can traps (Service 1993) in December 2004 and January 2005 in a neighborhood (Remedios de Escalada–31°20′14″ S, 64°10′39″ W) located in the northern area of Córdoba City. Four traps were used every night: 2 baited with a 3-week-old chicken (*Gallus gallus*; weight approximately 1.5 kg) each, and 2 containing 3 eared doves each. Traps were operated from 1800 to 0900 hours to ensure the collection of *Culex* mosquitoes. Birds were placed into the traps tied by beak and legs to restrain their movements and prevent them from eating mosquitoes. The next day mosquitoes were collected from the traps by manual aspirator, transported alive to the laboratory, and pooled by species, kind of bait, and engorged/not engorged.

As *Cx. quinquefasciatus* and *Cx. interfor* have epidemiological importance for SLEV transmission in Córdoba (Diaz et al. 2006), these 2 mosquito species were analyzed by a chi-square test to detect statistical differences between different kinds of baits in each mosquito species.

RESULTS

Viremia profiles and serology

Of the 30 inoculated eared doves, 24 developed a detectable viremia by plaque assay between the 1st and 7th dpi, but 60% exceeded the oral infectious dose 50 (OID50) for *Cx. quinquefasciatus* (2.9 $\log_{10}$ PFU/mL–genotype V SLEV 78V-6507 strain) (Mitchell et al. 1983). Titers ranged from 2 to 5.5 $\log_{10}$ PFU/mL, the highest being observed on the 4th dpi (Fig. 1). The highest percentages of birds with a detectable viremia occurred between the 2nd and 5th dpi (60%–90%). During the study, neither deaths nor clinical signs of illness were observed in the 30 inoculated eared doves. Although 6 of the inoculated eared doves did not develop detectable viremia, the 30 individuals showed neutralizing antibodies on the 14th dpi, with titers ranging from 1:40 to 1:160 (data not shown).

Mosquito host preferences

A total of 2792 mosquitoes belonging to 5 species were collected (Table 1). A mean of 46 mosquitoes per trap night were collected. *Cx. quinquefasciatus* was the most abundant species, representing 91% of the captures, from which 71% were collected in chicken-baited traps. Of the mosquitoes collected in eared dove-baited traps, 90% were *Cx. quinquefasciatus*. As a result of chi-square test, no relevant differences were observed between the 2 types of baits (chicken and eared dove) in either of the species (*Cx. quinquefasciatus* [Pearson's X^2 statistics = 0.86; df = 1; $p = 0.354$] or *Cx. interfor* [Pearson's X^2 statistics = 0.63; df = 1; $p = 0.426$]) analyzed.

DISCUSSION

All eared doves inoculated with the genotype V SLEV 78V-6507 strain developed viremia and/or neutralizing antibody. Mitchell et al. (1983) estimated a viremia for genotype V (78V-6507) of 2.9 $\log_{10}$ PFU/mL as the OID50 needed to infect *Cx. quinquefasciatus* mosquitoes. Experimentally inoculated eared doves in this work, with the same SLEV strain, developed viremias higher than the OID50 between the 1st and 7th dpi (Fig. 1). Mitchell et al. (1983) also detected a different OID50 for a different viral strain of SLEV (79V-2533–genotype III). In 2006, the genotype III SLEV strain was isolated during an outbreak. Eared doves also devel-

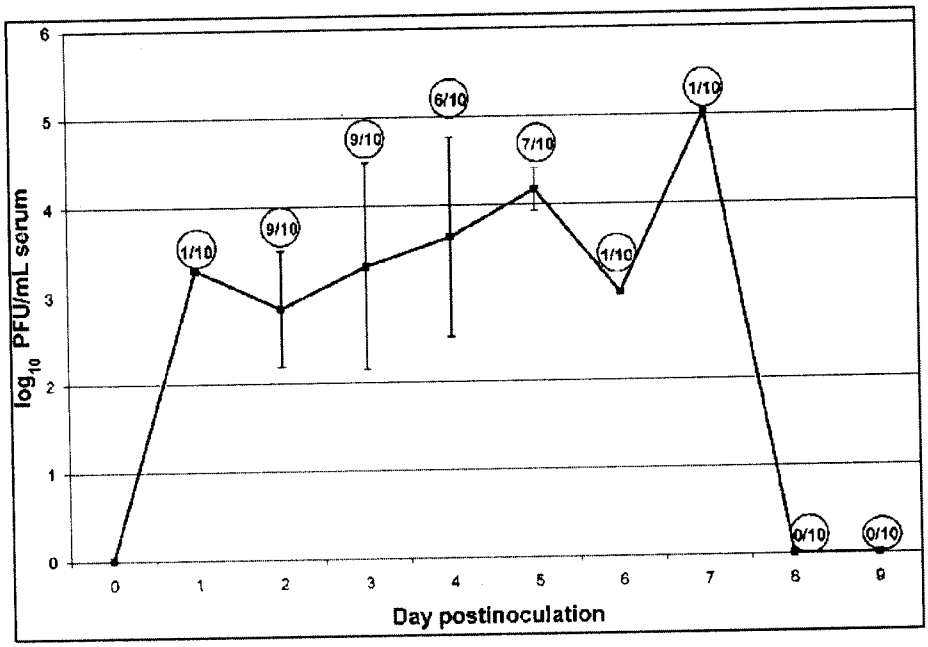

FIG. 1. Mean daily viremia titers for eared doves (*Z. auriculata*) inoculated with the genotype V 78V-6507 SLEV strain as a function of postinoculation days. Error bars represent standard deviations of the mean. Circles represent the number of viremic birds out of the number of birds bled per day.

oped higher viremias than the OID50 for genotype III (3.5 $\log_{10}$ PFU/mL) between the 3rd and 7th dpi. However, future assays should be carried out to elucidate the viremia profile for genotype III SLEV in eared doves.

In the United States, studies carried out with mourning doves (*Z. macroura*) demonstrated that adults were not competent hosts for SLEV, but nestling mourning doves were competent and produced enough viremias to infect mosquitoes (Mahmood et al. 2004). The difference with our eared dove could be related to genetic differences between both related dove species and distinct biological characteristics of the SLEV strains (Bowen et al. 1980).

The viremia profiles herein detected in eared doves were variable among individuals. Such differences could be based on genetic differences among individuals, sex, and age. Genetic differences detected in the same microrodent

TABLE 1. TOTAL NUMBER OF MOSQUITOES COLLECTED USING 2 CHICKEN (*GALLUS GALLUS*)- AND 2 EARED DOVE (*ZENAIDA AURICULATA*)-BAITED CAN TRAPS OPERATED DURING 15 NIGHTS IN REMEDIOS DE ESCALADA NEIGHBORHOOD (CÓRDOBA CITY) IN DECEMBER 2004 AND JANUARY 2005

	Baited can traps				
	Chicken		*Eared dove*		
Mosquito species	*E*	*NE*	*E*	*NE*	*Total*
Aedes aegypti	19	9	15	10	53
Culex apicinus	0	0	0	1	1
Cx. interfor	75	40	34	16	165
Cx. quinquefasciatus	1609	192	643	96	2540
Culex spp.	19	8	1	2	30
Ochlerotatus albifasciatus	3	0	0	0	3
Total	1725	249	693	125	2792

E, engorged; NE, not engorged.

Calomys musculinus population determined distinct viremia profiles for Junín virus (Sabattini and Contigiani, unpublished data). Although age does influence viremia, this factor was under control as all inoculated birds belonged to post hatch-year individuals. Therefore, all differences observed could be attributed to sex as a genetic difference among individuals. Follow-up studies should be done to evaluate this hypothesis.

In general, nestling birds are more susceptible to viral infection than adults. This difference was observed for both house sparrows and mourning doves experimentally infected with SLEV in the United States (Trent et al. 1980, Mahmood et al. 2004). Based on these results, it could be expected that nestling eared doves also develop higher viremia titers than adults, acting as amplifier hosts for SLEV. In Argentina, *Z. auriculata* and *Cx. quinquefasciatus* (potential vectors of SLEV in the country) are distributed in the same rural and urban areas (Bucher et al. 1981, Almirón and Brewer 1995), and the reproductive period for both species is the rainy-summer season (Murton et al. 1974, Diaz et al. 2003). At the least, the first *Cx. quinquefasciatus* abundance peak, during the warm-rainy season of the temperate Córdoba Province, may occur simultaneously with the eared dove breeding period, amplifying SLEV activity. However, laboratory assays using nestling, young, and adult eared doves are needed to determine if younger eared doves are better amplifier hosts for SLEV than adults.

The most abundant mosquito species collected in baited can traps were *Culex* spp. indicating their preference for birds. No statistically significant differences between the 2 kinds of baits used were found for any of the 2 mosquito species analyzed. However, eared doves were highly less attractive than chickens. The mosquito host preference studies indicated both *Cx. quinquefasciatus* and *Cx. interfor* were attracted by eared doves, and also included this bird species as a blood source. Since both species (*Cx. interfor* and *Cx. quinquefasciatus*) were found naturally infected with SLEV during an outbreak of encephalitis in humans (Diaz et al. 2006), they are deemed as a potential vectors for SLEV in the central region of Argentina.

The efficiency of an avian host depends upon several factors. The host must first be susceptible to the viral infection, it must develop a viremia titer higher than the minimum infection threshold needed to infect the mosquito vectors, it must then be bitten by the mosquito vectors and found naturally infected, and there must be an overlap in the geographic distribution of the virus, avian hosts, and mosquito vectors. Being abundant in the same period is important as well (McLean and Bowen 1980). Considering that eared doves were frequently found positive for antibodies against SLEV, that they developed viremia titers higher than OID50, that these were for long enough to infect *Cx. quinquefasciatus*, and that these mosquitoes also fed on eared doves, they could be considered competent avian hosts for SLEV.

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